Effect of Thyme Water Extract (0, 1 ml/30 ml buffered rumen fluid) on Short Chain Fatty Acid, Net Energy For Lactation, Metabolizable Energy and Organic Matter Digestibility of Soybean Meal Using In Vitro Gas Production Technique

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Abstract: This experiment was conducted to survey effect of adding different levels (0, 1 ml/30ml buffered rumen fluid) of Thyme water extract on soybean meal (SBM) degradability were studied by in vitro gas producing techniques. Gas production test with mixtures of filtered rumen liquid of three Taleshi native male cattle rumen in times of 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours were performed. Calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) of SM (81.21 g/kg DM, 12.67 MJ/kg DM and 1.24 mmol, respectively) were compared to 0.3 Thyme water extract (70.03 g/kg DM, 10.456 MJ/kg DM, and 0.968 mmol, respectively).


Keywords: metabolizable energy; soybean meal; gas production technique; organic matter digestibility

Abbreviations: SBM, soybean meal; EE, ether extract; ZM, Zataria multiflora; OMD, organic matter digestibility; ME, metabolizable energy, SCFA, short chain fatty acid; CP, crude protein; NEₙ, net energy for lactation.

1. Introduction

Ruminal microbial activity is essential for the use of structural carbohydrates and synthesis of high quality protein in ruminants. However, microbial fermentation in the rumen may result in considerable energy and protein losses as methane and ammonia (NRC, 2001; Bunthoeun, 2007).

Methane emission and excretion of N from ruminant livestock substantially implicate global warming and N pollution. Recently, there has been an increased interest in saponins or saponin-containing plants for modifying ruminal fermentation. Saponins are phytochemical compounds composed of a steroid or triterpennoid sapogenin linked to one or more sugar chains (Cheeke, 1999; Bunthoeun, 2007).

Saponins are widely found in plants and are generally grouped among anti-nutritional factors or may be toxic and cause photosensitization (Flaoyen and Wilkins, 1997; Meagher et al., 2001; Pirez et al., 2002; Bunthoeun, 2007). Recently, various findings indicate beneficial effects of saponins to animals and the environment in reducing methane produced by animals (Cheeke, 1996; Takahashi et al., 2000; Pen et al., 2006; Bunthoeun, 2007). Saponins have been shown to react with cholesterol present in membranes of eukaryotic cells but not of prokaryotic cells and therefore, decrease or eliminate protozoa in the rumen without inhibiting bacterial growth (Bunthoeun, 2007).

in vivo, in situ and in vitro methods have been used to evaluate the nutritive value of feedstuffs. The in vitro gas production technique has proven to be a potentially useful technique to evaluate the nutritive value of feedstuffs, since it gives an estimate of the potential rate and extent of nutrient fermentation in the rumen. However, this technique is measuring gas produced by the fermentation of energy containing components in feeds, and not only that of protein (Mirzaei-Aghsaghal et al., 2008a, 2008b); (Maheri-Sis et al., 2007, 2008); (kiyani et al., 2010).

The objective of this study was to evaluate the potential of natural plant extracts as fermentation pattern in vitro gas production characteristics, organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA) and net energy for lactation (NEₙ) by in vitro gas production technique.

2. Material and Methods

2.1. Thyme Samples

During summer season Thyme samples were collected from different parts of Esfahan province. Next, there were drying for one week, and
homogeneous mixture were papered for nutritive chemical analyzes. For determination of (Thyme) effects, we added Thyme extracts with two levels (0 1 mL: 200 mg samples) into gas test syringes. All samples were then ground in a laboratory mill through a 1 mm screen.

2.2. Procedure of plant extracts preparation

The plant extracts were prepared according to (Patra et al., 2006) with some modifications. The plant materials were dried at 50°C and ground in mills to pass a 1 mm sieve and 100 g placed in 1000 ml of distilled water solvent. The flasks of all the solvents were stoppered and agitated with a magnetic stirrer for 24 h at room temperature. Then the solutions were centrifuged at 3000 g for 10 min. The residue was re-extracted with 500 ml of distilled water for 24 h stirring at room temperature and centrifuged again at 3000 g for 10 min. The plant extracts were combined. Distilled water was evaporated from the solution at approximately 85°C by using a rotary-evaporator (Patra et al., 2006; Sallam et al., 2009).

2.3 Treatments and experimental design

The different levels of thyme water extract were added to the diet sample. Tow levels (0 and 1 ml/30 ml buffer rumen fluid) of thyme water extract were investigated as follow: (i) no additive; (ii) thyme water extract.

2.4. In vitro gas production

Fermentation of soybean meal samples were carried out with rumen fluid was obtained from three fistulated Taleshi native male cattle fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of (Menke and Steingass, 1988) as follows. 200 mg dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml in the absence and presence of level 1 ml (Thyme water extract). The syringes were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C.

The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for soybean meal samples were determined by subtracting the volume of gas produced in the blanks. Cumulative gas production data were fitted to the model of (Ørskov and McDonald 1979).

\[ P = a + b (1-e^{-ct}) \]

Where P is the gas production at time t, a the gas production from soluble fraction (ml/200mg DM), b the gas production from insoluble fraction (ml/200mg DM), c the gas production rate constant (ml/h), a + b the potential gas production (ml/200mg DM) and t is the incubation time (h).

The metabolizable energy (MJ/kg DM) content of soybean meal was calculated using equations of (McDonald et al., 1995), (Menke and Steingass1988) and (Menke et al. 1979) as follows:

for all feeds,

\[ ME (MJ/kg DM) = 0.016 \text{ DOMD} \]

for forage feeds,

\[ ME (MJ/kg DM) = 2.20 +0.136 \text{ GP} + 0.057 \text{ CP} +0.0029 \text{ CF}^2 \]

For concentrate feeds,

\[ ME (MJ/kg DM) = 1.06 +0.157 \text{ GP} +0.084 \text{ CP} +0.22 \text{ CF} -0.081 \text{ CA} \]

Where:

\[ \text{GP} = \text{The 24 h net gas production (ml/200 mg)}^{-1} \]
\[ \text{CP} = \text{Crude protein} \]

Short chain fatty acids (SCFA) are calculated using the equation of (Makkar 2005) and (Maheri-Sis 2007, 2008).

\[ \text{SCFA (mmol)} = 0.0222 \times \text{GP} - 0.00425 \]

The organic matter digestibility was calculated using equations of (Menke et al. 1979) as follows:

\[ \text{OMD (g/kg DM)} = (%)14.88 +0.889 \text{ GP} +0.45 \text{ CP} +\text{XA} \]

Where:

\[ \text{GP} = \text{About 24 h net gas production (ml/200 mg)}^{-1} \]
\[ \text{CP} = \text{Crude protein (％)} \]
\[ \text{XA} = \text{Ash content (％)} \]

\[ \text{NEL (MJ/kg DM)} = 0.115 \times \text{GP} + 0.0054 \times \text{CP} + 0.014 \times \text{EE} - 0.0054 \times \text{CA} - 0.36 \]

2.5. Statistical Analysis

Data on apparent gas production parameters were subjected to one-way analysis of variance using the analysis of variation model ANOVA of SAS (2000). Multiple comparison tests used Duncan’s multiple-range test (1980).

Significance between individual means was identified using the Duncan’s multiple range tests. Mean differences were considered significant at (P<0.05). Standard errors of means were calculated from the residual mean square in the analysis of variance. All data obtained from three replicates n=3.
3. Results

3.1. In vitro gas production

Calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) of soybean meal (SBM) are presented in Table 1.

Calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) of SBM (81.21 g/kg DM, 12.67 MJ/kg DM and 1.24 mmol, respectively) were compared to 0.3 Thyme water extract (70.03 g/kg DM, 10.456 MJ/kg DM, and 0.968 mmol, respectively).

Table 1

<table>
<thead>
<tr>
<th>Estimated parameters</th>
<th>OMD</th>
<th>ME</th>
<th>SCFA</th>
</tr>
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<tbody>
<tr>
<td>i</td>
<td>81.21</td>
<td>12.67</td>
<td>1.24</td>
</tr>
<tr>
<td>ii</td>
<td>70.03</td>
<td>10.456</td>
<td>0.968</td>
</tr>
</tbody>
</table>

OMD: organic matter digestibility (g/kg DM), ME: metabolisable energy (MJ/kg DM), SCFA: short chain fatty acid (mmol)

4. Discussions

There was a positive correlation between NFC content of feeds and gas production, but feed CP, NH₃-N and NDF levels were negatively correlated with gas production (Getachew et al., 2004; Maheri-Sis et al., 2007). Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds. Variation in chemical components of feeds such as starch, NFC, OM, CP, NDF and soluble sugars contents can be result in variation of in vitro gas production volume (Maheri-Sis et al., 2008).

This study suggested that the thyme water extract have the potential to affect ruminal fermentation efficiency, and be a promising methane mitigating agent.

Salamat azar et al. (2011a) evaluation effects of addition three doses zataria multiflora water extract (0.3 ml/30 ml buffered rumen fluid) on the short chain fatty acid, net energy, metabolizable energy and organic matter digestibility of canola meal using in vitro gas production technique and report amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NEI) contents of canola meal (79.46 g/kg DM, 10.27 MJ/kg DM, 1.046 mmol and 5.28 MJ/kg DM, respectively) were high as compared to zataria multiflora water extract (0.3 ml/30 ml buffered rumen fluid) were (41.85 g/kg DM, 3.63 MJ/kg DM, 1.047 mmol and 1.22 MJ/kg DM, respectively). These results are in agreement with the findings of Salamat azar et al. (2011a, b).

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